



UNIVERSITI PUTRA MALAYSIA

**INDUCTION STRATEGIES FOR INTERFERON- α 2B PRODUCTION IN
PERIPLASMIC SPACE OF *ESCHERICHIA COLI***



SITI NOR ANI BINTI AZAMAN

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PERIPLASMIC SPACE OF *ESCHERICHIA COLI***

By

SITI NOR ANI BINTI AZAMAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

INDUCTION STRATEGIES FOR INTERFERON- α 2B PRODUCTION IN PERIPLASMIC SPACE OF *ESCHERICHIA COLI*

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SITI NOR ANI AZAMAN

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Chair: Assoc. Prof. Mohd Puad Abdullah, PhD

Faculty: Institute of Bioscience

Interferon- α 2b (IFN- α 2b) is a member of cytokine family with the ability to induce antiproliferative, antiviral, antineoplastic and immunomodulating activities. It is frequently used in the treatments of gastrointestinal tract diseases, various cancers and chronic hepatitis B. Due to its diagnostic and therapeutic potentials the production of IFN- α 2b in large quantities is required to meet the demands from research, clinical and industrial applications. This study was carried out to enhance the production of recombinant IFN- α 2b in the periplasmic space of *E. coli* using the recombinant *E. coli* Rosetta-gami 2(DE3) harboring the plasmid pET26b-IFN- α 2b. In this study, different induction parameters (temperature, IPTG concentration, point of induction and post induction time) were analyzed and optimized during induction for optimal periplasmic IFN- α 2b production. Further analysis was done on the effect of pH medium on IFN- α 2b production in different fractions.

Preliminary screening performed on four induction parameters shows that only three parameters (temperature, IPTG concentration and point of induction) improved the

IFN- α 2b production. These parameters were optimized individually by narrowing the working range as follows: temperature from 16°C to 30°C, IPTG lower than 2 mM, and induction point between early-log phase and mid-log phase on the growth curve of *E. coli*. A response surface methodology (RSM) based on a central composite design was used to optimize the induction parameters. The proposed induction strategy consisted of three optimized parameters: i) induction point at A_{600} of 4, ii) IPTG strength at 0.05 mM, and iii) induction temperature at 25°C. The strategy yielded 1.21 $\mu\text{g/mL}$ of IFN- α 2b, which represents 82% of the soluble IFN- α 2b that was successfully transferred to the periplasmic space of *E. coli* after 18 h of induction.

A study on the effect of pH on IFN- α 2b production in different fractions reveals that the highest yield was obtained at pH 7 with a high buffering capacity (0.2 M). At this pH, a higher IFN- α 2b production was obtained in the periplasmic fraction. Whereas, the amount of inclusion bodies formed was reduced. This was evident by the lack of aggregated materials in the cytoplasm of *E. coli* observed under the electron microscope. This study also proved that the suitable buffering capacity would maintain the pH of media during fermentation.

Finally, when the optimized induction parameters with the appropriate media pH was applied, the total IFN- α 2b obtained was 1.43 $\mu\text{g/mL}$, a 86.9-fold increase in productivity compared to the IFN- α 2b produced under non-optimal condition (16.26 ng/mL). Thus, proper optimization of fermentation conditions was proved useful to improve the production of periplasmic IFN- α 2b in *E. coli*.

Abstrak tesis untuk dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**STRATEGI INDUKSI UNTUK PENGHASILAN INTERFERON- α 2B DI
DALAM PERIPLASMIK *ESCHERICHIA COLI***

Oleh

SITI NOR ANI AZAMAN

Januari 2011

Pengerusi: Prof. Madya Mohd Puad Abdullah, PhD

Fakulti: Institut Biosains

Interferon- α 2b (IFN- α 2b) ialah protein yang tergolong dalam kumpulan sitokin yang mempunyai kebolehan untuk menginduksi aktiviti anti-penggunaan, anti-virus, anti-neoplastik dan imunomodulasi. Ia sering digunakan dalam rawatan penyakit seperti penyakit saluran pencernaan, pelbagai jenis kanser dan hepatitis B kronik. Berdasarkan potensinya dalam bidang diagnostik dan terapeutik, pengeluaran IFN- α 2b diperlukan bagi memenuhi permintaan dalam sektor penyelidikan, klinikal dan perindustrian. Kajian ini dijalankan untuk meningkatkan penghasilan rekombinan IFN- α 2b di dalam ruang periplasmik *E. coli* menggunakan *E. coli* rekombinan Rosetta-gami 2 (DE3) yang mengandungi plasmid pET26b-IFN- α 2b. Dalam kajian ini, beberapa parameter induksi (suhu, kepekatan IPTG, masa induksi dan tempoh induksi) dianalisis dan dioptimumkan untuk penghasilan IFN- α 2b yang optimum. Analisis selanjutnya telah dilakukan ke atas kesan pH media ke atas penghasilan IFN- α 2b di dalam bahagian yang berlainan.

Penyaringan awal ke atas empat parameter induksi menunjukkan hanya tiga parameter induksi (suhu induksi, kepekatan IPTG dan masa induksi) dapat meningkatkan penghasilan IFN- α 2b. Ketiga-tiga parameter ini dioptimumkan secara individu dengan mengecilkan julat kerja seperti berikut: suhu induksi daripada 16°C hingga 30°C, kepekatan IPTG kurang daripada 2 mM, dan masa induksi antara fasa awal-log hingga fasa pertengahan-log pada lengkung pertumbuhan *E. coli*. Kaedah respon permukaan (RSM) berdasarkan reka bentuk komposit pusat telah digunakan untuk mengoptimumkan parameter induksi. Strategi induksi yang dicadangkan mengandungi tiga parameter optimum: i) masa induksi pada A_{600} 4, ii) kepekatan IPTG pada 0.05 mM, dan iii) suhu induksi pada 25°C. Strategi induksi yang optimum telah menghasilkan 1.21 $\mu\text{g/mL}$ IFN- α 2b, yang mewakili 82% daripada IFN- α 2b larut yang berjaya dipindahkan ke ruang periplasmik *E. coli* selepas 18 jam induksi.

Kajian mengenai kesan pH ke atas penghasilan IFN- α 2b di dalam pecahan yang berlainan membuktikan bahawa hasil yang paling tinggi telah diperolehi pada pH 7 dengan menggunakan kapasiti penimbal yang tinggi (0.2 M). Pada pH ini, penghasilan IFN- α 2b yang tinggi telah diperolehi daripada fraksi periplasmik dengan pengurangan jumlah pembentukan jasad rangkuman. Ini dibuktikan dengan berkurangnya molekul agregat di ruang sitoplasmik *E. coli* yang diperhatikan di bawah mikroskop elektron. Kajian ini juga membuktikan penggunaan kapasiti penimbal yang sesuai dapat mengawal pH media semasa fermentasi.

Akhirnya, apabila parameter induksi yang telah dioptimumkan digunapakai dengan pH media yang sesuai, jumlah keseluruhan IFN- α 2b yang diperolehi ialah 1.43 $\mu\text{g/mL}$,

86.9 kali ganda peningkatan dari segi produktiviti berbanding IFN- α 2b yang dihasilkan pada keadaan tidak optimum (16.26 ng/mL). Maka, pengoptimuman keadaan fermentasi yang terbaik telah terbukti berguna bagi meningkatkan penghasilan periplasmik IFN- α 2b dalam *E. coli*.

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I certify that a Thesis Examination Committee has met on **14 January 2011** to conduct the final examination of Siti Nor Ani Azaman on her thesis entitled “**Induction Strategies for Interferon- α 2b Production in the Periplasmic Space of *Escherichia Coli***” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A)] 15 March 1998. The Committee recommends that the student be awarded the degree of Master Science.

Members of the Thesis Examination Committee were as follows:

Shuhaimi Mustafa, PhD

Associate Professor
Halal Products Research Institute
Universiti Putra Malaysia
(Chairman)

Nor ‘Aini Abdul Rahman, PhD

Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Umi Kalsom Md Shah, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Aidil Abdul Hamid, PhD

Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mohd Puad bin Abdullah, PhD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

University Putra Malaysia

(Chairman)

Arbakariya bin Ariff, PhD

Professor

Faculty of Biotechnology and Biomolecular Sciences

University Putra Malaysia

(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

SITI NOR ANI AZAMAN

Date: 14 January 2011



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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xviii
CHAPTER	
1 INTRODUCTION	1
1.1 Overview	1
1.2 Objectives	3
2 LITERATURE REVIEW	4
2.1 Interferon- α 2b	4
2.1.1 Introduction	4
2.1.2 Properties of Interferon- α	5
2.1.3 Application of Interferon- α	9
2.2 Production of Recombinant Interferon- α	10
2.3 <i>Escherichia coli</i> Expression System	12
2.3.1 Introduction	12
2.3.2 <i>E. coli</i> structure and protein targeting	13
2.4 Production of recombinant Interferon- α 2b in <i>E. coli</i>	22
2.5 Inclusion bodies	24
2.6 Effect of fermentation parameters on recombinant protein production	27
2.6.1 Effect of temperature	27
2.6.2 Effect of IPTG concentration	28
2.6.3 Effect of induction point	29
2.6.4 Effect of post-induction time	30
2.6.5 Effect of culture pH	31
2.7 Experimental design for parameter optimization	33
2.7.1 Introduction	33
2.7.2 Use of response surface methodology (RSM) in fermentation	34
2.8 Concluding remarks	38

3	SCREENING OF OPTIMAL INDUCTION PARAMETERS FOR PRODUCING INTERFERON-α2B IN THE PERIPLASMIC SPACE OF <i>ESCHERICHIA COLI</i>	39
3.1	Introduction	39
3.2	Materials and methods	42
3.2.1	<i>E. coli</i> strain	42
3.2.2	Medium	42
3.2.3	Fermentation condition	42
3.2.4	Experimental design	43
3.2.5	Determination of bacterial cell density	46
3.2.6	Protein extraction from periplasm	46
3.2.7	Determination of total protein concentration	47
3.2.8	Determination of IFN- α 2b concentration	48
3.3	Results	49
3.3.1	Effect of IPTG concentration on PrIFN- α 2b production	49
3.3.2	Effect of induction point on PrIFN- α 2b production	52
3.3.3	Effect of induction temperature on PrIFN- α 2b production	54
3.3.4	Post-induction time	56
3.4	Discussion	60
3.5	Conclusion	64
4	OPTIMIZATION OF INDUCTION STRATEGY FOR IMPROVED INTERFERON-α2B PRODUCTION IN THE PERIPLASM OF <i>ESCHERICHIA COLI</i> USING RESPONSE SURFACE METHODOLOGY	65
4.1	Introduction	65
4.2	Materials and methods	68
4.2.1	<i>E. coli</i> strain	68
4.2.2	Medium	68
4.2.3	Fermentation condition	68
4.2.4	Optimization using response surface methodology	69
4.2.5	Determination of suitable post-induction time for optimized induction strategy	70
4.2.6	Protein extraction from periplasm	71
4.2.7	Protein extraction from cytoplasm	71
4.2.8	Determination of total protein concentration	71
4.2.9	Determination of IFN- α 2b concentration	72
4.2.10	Transmission electron microscope analysis	72
4.3	Results	73
4.3.1	Experimental run and statistical analysis	73
4.3.2	Effect of interaction parameters on the production of PrIFN- α 2b and percentage of soluble IFN- α 2b translocated to the periplasmic space of <i>E. coli</i>	81

4.3.3	Validation	86
4.3.4	Identification of suitable post-induction time for optimized induction strategy	90
4.4	Discussion	91
4.5	Conclusion	95
5	EFFECT OF INITIAL CULTURE pH ON PERIPLASMIC INTERFERON-α2B PRODUCTION AND CELLULAR STRUCTURE OF <i>ESCHERICHIA COLI</i>	96
5.1	Introduction	96
5.2	Materials and methods	99
5.2.1	<i>E. coli</i> strain	99
5.2.2	Culture media	99
5.2.3	Fermentation condition	99
5.2.4	Experimental design	100
5.2.5	Protein extraction from periplasm	100
5.2.6	Protein extraction from cytoplasm	101
5.2.7	Protein solubilization from inclusion bodies fraction	101
5.2.8	Determination of IFN- α 2b concentration	101
5.2.9	Transmission electron microscope analysis	101
5.3	Results	102
5.3.1	pH range for <i>E. coli</i> growth	102
5.3.2	Effect of culture pH on IFN- α 2b production in different cellular spaces of <i>E. coli</i>	104
5.3.3	Effect of culture pH on structure and cellular component of <i>E. coli</i>	106
5.4	Discussion	108
5.5	Conclusion	111
6	SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	112
6.1	Summary and general conclusion	112
6.2	Recommendation for future research	114
	REFERENCES	115
	APPENDICES	130
	BIODATA OF STUDENT	144
	LIST OF PUBLICATIONS	145